Observational Study

Phytochemical screening and antibacterial activities of Amaranthus viridis, Cynodon dactylon & Aerva sanguinolanta — A preliminary investigation

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Dental caries is the most common microbial disease of human being. Prevention of dental caries have been attempted in various form including use of natural phytochemicals isolated from plants. So in the present study in-vitro, qualitative, phytochemical screening of three known medicinal plants ie. Amaranthus viridis, Cynodon Dactylon, Aerva sanguinolanta were performed. The result reveal the presence of alkaloids, cardiac glycoside, flavonoids, carbohydrate, protein, fat and steroid in methanolic extract of Cynodon dactylon, alkaloids & flavonoids in methanolic extract of Amaranthus viridis and Flavonoid in methanolic extract of Aerva sanguinolanta.

The present study also determined the anti-microbial activity of the three same plant extracts on a specific cariogenic bacteria Streptococcus mutans (MTCC -890). The 100% & 50% methanol extract of Amaranthus viridis, Cynodon dactylon & Aerva sanguinolanta shows inhibition zone of (12.75±0.50 mm & 11.62±0.86 mm), (20.48±0.49 mm &11.62±0.86 mm), (6.97±0.73 mm & no inhibition) respectively in disc diffusion assay.

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Key words **: Antibacterial assey, dental caries, medicinal plants, phyto chemical screening.**

Dental caries is one of the most common oral diseases worldwide with a prevalence of up to 90% in school age children & the majority of the adults are affected[1]. Specific types of acid-producing bacterias, including Streptococcus mutans, colonize the dental surface and cause demineralization of the hard tooth structure in the presence of fermentable carbohydrates leading to cavitation[2]. Prevalence of dental caries may be reduced by controlling oral microbes causing dental caries.

There have been numerous reports of traditional medicinal plant extracts or phytochemicals that have been shown to inhibit the growth of oral pathogens, and reduce the symptoms of oral diseases specially dental caries. So the aim of the present study is in-vitro, qualitative, phytochemical screening and antibacterial assey of three plant specimen Amaranthus viridis, Cynodon dactylon & Aerva sanguinolanta against a specific cariogenic bacteria Streptococcus mutans (MTCC-890) .

Amaranthus viridis is commonly known as Green Amaranth (Engl.) Bauan (Bon.) note in Bengali, is an erect, smooth, branched, unarmed herb. Traditionally used as anti-inflammatory, diuretic, analgesic, antiulcer, antiemetic, laxative. It is also considered antiproliferative, antifungal, antiviral.

Cynodon dactylon also known as dûrvâ grass in Bengali, Bermuda grass in English, is hard to eradicate weed, highly desirable turf grass and able to survive climate of heat and drought. Traditionally apply crushed leaves to minor wounds as a styptik to stop bleeding.

The plant Aerva sanguinolenta family Amaranthaceae, is an erect or rambling perennial herb found in tropical countries of Asia. In Bengali it is called 'Chaya'. In folklore medication leaf and flower of the plant were used as wound healing and anti inflammatory. The whole plant was used as diuretic and have shown antimicrobial activity.

Material and Method

(1) Phytochemical Evaluation of Plant Extracts :

(a) Collection, authentication & pre-treatment of plant sample — Plants with mature leaves and seeds of Amaranthus viridins, Cynodon dactylon & Aerva sanguinolanta were collected from medicinal garden of Ramkrishna Mission Ashram, Kolkata, West Bengal during the month of July 2017, were authenticated by the taxonomist from Botanical Survey of India, West Bengal. The plant samples were washed, shade dried, chopped, made into a coarse powder in a mixer grinder (Philips HL) and the coarse powder were stored in sealed, labelled polythene packet.

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(b) Preparation of plant extract — The coarse powder (100g each) of the three selected plant specimen were subjected to hot continuous extraction with 300 ml methanol (Mercks) by Soxhlet apparatus (Borosil) followed by distillation using rotary evaporator (RE100PR0MFGD silicogex/USA Takashi) with an yield of 2 gm extract by weight which were stored in sterilized glass beaker in a refrigerator at 4 degree centigrade.

(c) Qualitative phytochemical screening of plant sample — Phytochemical screening for the presence of Tannins, alkaloids, glycosides, flavonoids, carbohydrate, steroid, protein, fat, cardiac glycoside and triterpinoid were performed for the three plant extracts using specific procedures as mentioned in Table 1.

Table 1 — *Result of qualitative phytochemical screening of methanolic extract of Cynodondactylon, Amaranthusviridis & Aervasanguinolenta*

		Phytochemical screening of *Cynodondactylon*		Phytochemical screening of *Amaranthus viridis*		Phytochemical screening of *Aerva sanguinolenta*	
		Positive	Negative	Positive	Negative	Positive	Negative
Phyto constituents	Phyto chemical test		✔		✔		✔
Test for Tannin	Ferric Chloride test		✔		✔		✔
	Gelatin test		✔		✔		✔
Test for Alkaloid	Mayer's test	✔		✔			✔
	Wagne's test	✔	✔	✔	✔		✔
Test for Flavonoid	Ferric Chloride test	✔			✔	✔	
	Shinoda Test		✔		✔		✔
	ZnHCL acid reduction test		✔		✔		✔
Test for Saponin Glycoside	Foam test	✔			✔		✔
Test for Carbohydrate	Molisch's test	✔			✔		✔
Test for Steroid	Salkowaski test		✔		✔		✔
Test for Triterpinoid	Salkowaski test		✔		✔		✔
Test for Protein	Xanthoproteic test	✔			✔		
	Biuret test		✔		✔		✔
	Ninhydrin test	✔			✔		✔
	Test for Amino acid	✔			✔		✔
Test for Cardiac Glycoside			✔		✔		✔
Test for Fat			✔		✔		✔

(2) Antibacterial assay of plant extracts :

(a) Preparation of different concentration of plant extracts — Pre-measured amount of the three stored plant extracts (100 mg), each were mixed with fixed volume (1ml) of methanol solvents using electrical stirrer (Remi Laboratory Instruments) to prepare 100 mg/ml concentration mix which were kept as stock solution. For each plant extract two different solution (100% & 50%) with DMSO was prepared.

(b) Collection & Revival of bacterial sample — Freeze dried standard culture samples of Streptococcus mutans (MTCC -890) obtained from the Microbial Type Culture Collection and Gene Bank, Chandigarh. The stored bacterial sample was inoculated on Brain heart infusion broth (Himedia, Mumbai) , was incubated at 37°C for 24 hour. On the next morning turbidity appear on the broth.

(c) Screening for antimicrobial activity of plant products — Antimicrobial activity of 100% and 50% concentration of ethanolic extracts of the three plant products were measured by Disc diffusion method. Antibiotic discs (Himedia, India) Vancomycin 30% were used as positive control, 100% methanol is used as negative control. The plates were incubated for 24 hour at 37ºC. After the incubation, zones of inhibition appeared as a clear, circular halo surrounding the discs which was measured with the help of a sterile varnier caliper. The tests were repeated three times to overcome any technical errors that might occur during a single attempt.

Result

Qualitative phytochemical screening reveal the presence of alkaloids, cardiac glycoside, flavonoids, carbohydrate, protein, fat and steroid in methanolic extract of Cynodon dactylon, alkaloids & flavonoidsin methanolic extract of Amaranthus viridis and Flavonoid in methanolic extract of Aerva sanguinolanta as summarized in Table 1.

Result of Antimicrobial assey—The 100% & 50% methanol extract of Amaranthus viridis, Cynodon dactylon & Aerva sanguinolanta shows inhibition zone of (12.75±0.50 mm & 11.62±0.86 mm), (20.48±0.49 mm & 11.62±0.86 mm), (6.97±0.73 mm & no inhibition) respectively in disc diffusion assay against freeze dried standard culture samples of Streptococcus mutans (MTCC-890) Table 2.

Discussion

Medicinal plants contain some organic compounds which provide definite physiological action on the human

Table 2 — *Distribution of mean inhibition zone (in mm) of different concentrations of Cynodondactylon, Amaranthus viridis and Aerva sanguinolenta on Streptococcus mutans*

Concentration	Methanolic plant extract			Vancomycin (n=5) Conc. 30%
	Cynodondactylon plant extract (n=5)	Amaranthus viridis plant extract (n=5)	Aerva sanguinolenta plant extract (n=5)	
	Mean ± SD inhibition zone (in mm)			
50%	18.39±0.76	11.62±0.86	No inhibition	21.39±0.71
100%	20.48±0.49	12.75±0.50	6.97±0.73	

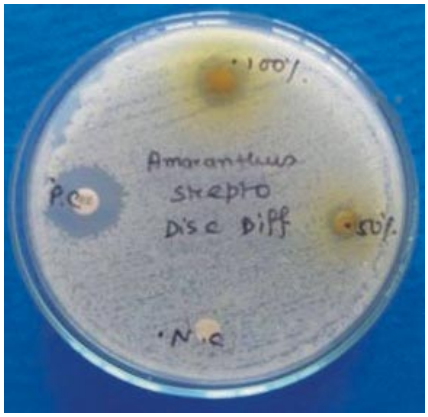

Fig 1 — The whole plant methanolic extract of Amaranthus viridis showed considerable antimicrobial activities in disc diffusion assay against Freeze dried standard culture samples of Streptococcus mutans (MTCC-890)

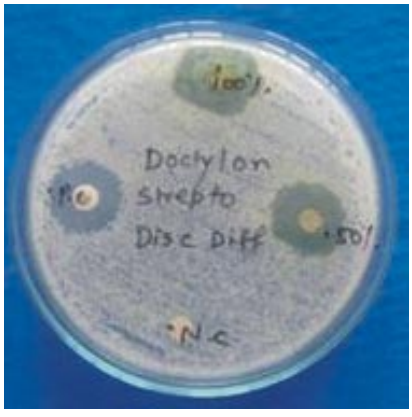

Fig 2 — The whole plantmethanolic extract of Cynodondactylon showed considerable antimicrobial activities in disc diffusion assay against Freeze dried standard culture samples of Streptococcus mutans (MTCC-890)

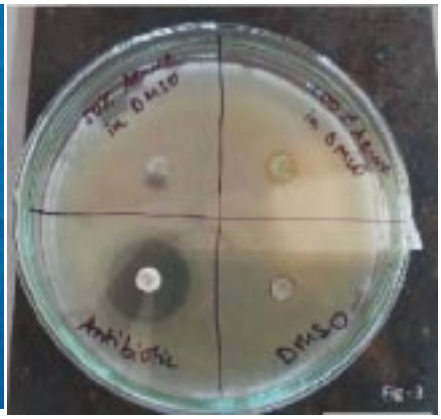

Fig 3 — The whole plant methanolic extract of Aerva sanguinolanta extracts showed considerable antimicrobial activities in disc diffusion assay against Freeze dried standard culture samples of Streptococcus mutans (MTCC-890)

body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids[3].

Phytochemical screening of Cynodon dactylon by Smitha KR *et al* revealed the presence of tannin, anthraquinone, phenol and alkaloid in the methanolic extract and steroid in the chloroformic extract. Aqueous extract showed the presence of tannin, saponin, flavonoid and alkaloid in aqueous extract[4] whereas the present study reveal presence of alkaloids, flavonoid, carbohydrate, steroid, protein, Cardiac glycoside in the methanolic extract of the plant.

In the present study whole plant methanolic extract of Amaranthus viridis revealed the presence of alkaloids & flavonoid SA Ahmed *et al* in their study also estimated presence of alkaloids, tannins, saponins and glycosides in both leaf and seed, methanolic extract of Amaranthus viridis[5].

Sampad *et al* in 2015 found out the flavonoid content of ethanolic and aqueous extract of Aerva sanguinolenta leaves to be 11.17±0.005mg and 3.53± 0.525mg of quarcetin equivalent per gram of extract respectively[6]. In the present study whole plant ethanolic extract of Amaranthus viridis revealed the presence of flavonoid .

In plants, the secondary metabolites have many beneficial effects including antibacterial and antiviral effects (Lipkin *et al*, 2004)[7].

Antimicrobial activity of methanolic extract of Amaranthus viridis, Cynodon dactylon & Aerva sanguinolanta against freeze dried standard culture samples of Streptococcus mutans (MTCC-890) were measured by disc diffusion method and the result were shown in Table 2. In 100% methanolic extract of all the three plants show better antibacterial activity than their 50% concentration. Both the higher & lower concentration of the three plants extracts show less antibacterial activity than the positive control-Vancomycin (30%). The current results support the earlier findings which demonstrate the presence of antimicrobial activity in seeds of Amarathaceae[7,8]. Iqbal *et al* tested the A. viridis leaf and seed extracts individually against a panel of microorganisms (locally isolated), including two bacteria, Staphylococcus aureus and Escherichia coli and found considerable antimicrobial activity against all the strains tested, particularly against Gram-positive bacterium like the present study. Smitha KR *et al* also tested the antibacterial activity of the extracts from C dactylon by well diffusion method. In their study methanol extract of C dactylon was found to be effective against Bacillus thuringiensis, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi and Escherichia coli[9]. GV Rao *et al* 2012 examined the methanolic extract of Aerva sanguinolenta Blume & its isolated compound bakuchiol for anti-microbial property and found out that they were active against gram positive organism.

Conclusion

From the present study it can be concluded that the plant species Amaranthus viridis, Cynodon dactylon & Aerva sanguinolanta had a rich amount of valuable phytoconstituents & both the plant extracts show effective antibacterial activity against Freeze dried standard culture samples of Streptococcus mutans (MTCC -890).

A further study on isolation of individual phytoconstituents from these plants and their antibacterial role against multiple cariogenic bacteria can establish their role in prevention of dental caries.

References

1 Petersen PE, Bourgeois D, Ogawa H, Estupinan S Day, Ndiaye C — The global burden of oral diseases and risks to oral health. *Bulletin of the World Health Organization* 2005; **83:** 661-9.

2 Nomura Y, Tsuge S, Hayashi M, Sasaki M, Yamaguchi J, Veda N, Hanada N — A survey on the risk factors for the preva-

lence of dental caries among preschool children in Japan. *Paediatr Dent J* 2004; **14:** 79-85
3 Edoga HO, Okwu DE, Mbaebie BO — Phytochemicals constituents of some nigerian medicinal plants. *Afr J Biotechnol* 2005; **4:** 685-8.
4 Smitha KR, Ruveena TN, RiniArif, Mesna A — Exploring antimicrobial and antioxidant activity of a traditional herbal medicine, Cynodondactylon (l.) Pers and its phytochemical screening. *World Journal of Pharmacy and Pharmaceutical Sciences* 2014; **3:** 1744-57.
5 Ahmed SA, Hanif S, Tehreemaiftkhar — Phytochemical profiling with antioxidant and antimicrobial screening of Amaranthusviridis l. Leaf and seed extracts. *Open Journal of Medical Microbiology* 2013; **3:** 164-71.
6 Iqbal JM, Hanif S, Mahmood Z, Anwar F, Jamil A — Antioxidant and antimicrobial activities of chowlai (amaranthusviridis l.) Leaf and seed extracts. *Journal of Medicinal Plants Research* 2012; **6:** 4450-5.
7 Lipkin A, Veronika A, Aleksandra N, Aleksey B, Eberhardt K, Mikhae B, *et al* — An antimicrobial peptide ar-amp from amaranth (amaranthusretroflexusl.)Seeds. *J Sci Direct* 2004; **34:** 93-5.
8 Cai YZ, Sun M, Croke H — Characterization and application of betalian pigment of plant amaranthaceae. *Trend in Food and Science Technology* 2005; **16:** 370-6.
9 Enzo A Palombo —Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases, Evidence-Based Complementary and Alternative Medicine 2011; Article ID 680354: 15.
10 Rao GV — Isolation and characterization of a potent antimicrobial compound from Aervasanguinolenta Blume.: An alternative source of Bakuchiol. *Journal of Pharmacy Research* 2012, **5:** 174-6.